

#### Day -1

1. Grow overnight culture in brain-heart infusion media (5 mL is sufficient, more is ok)

#### Day 1

1. Measure OD600 of overnight by diluting overnight culture 1:10
2. Inoculate this overnight into a new culture such that the total volume is 25 mL at OD600 = 0.05
  - a. Make a culture for negative control (dH<sub>2</sub>O, or MBP)
  - b. Make a culture for positive control (Amp, Kan)
  - c. Make a culture for sample (add protein)
3. Add appropriate additive to each culture
4. Measure OD600 at 30 minutes - 1 hour intervals, for 4-5 hours minimum
5. Repeat experiment in triplicates